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Dear Francis,

I have been so busy trying to write up the crystal structure work that I have neglected everything else, including replying to your letters. I had hoped to have a first draft of the paper (for Nature) ready for John Finch to show you at Cold Spring Harbor. All the first part is written, but the discussion is still incomplete. I intended to finish off with the harder parts of my memo of 19 May, which I now realise you may not have received before you left La Jolla. Some further thoughts are included in an addendum of 30 May, copy herewith. I would be glad to have your views as to how much should be put into the discussion,

I think the interpretation of Len's cutting pattern should go in, but what about going on to discuss the linkage number in the light of $1\frac{3}{4}$ turns per nucleosome particle. This implies that the screw in solution and on the particle must be different.

In reply to the question of yours on this last point, I know of no evidence which could disprove the statement that the number of bases per turn in solution is $10\frac{1}{2}$. I have discussed the whole matter with Michael and he may have written to you about this. Briefly, Bram has always maintained that the 12 Å peak in solution is at a different spacing from that in the fibre pictures, and this is borne out by Steve Harrison who has repeated these measurements and similar results can also be found in the paper by Maniatis and Lerman on ψDNA. The difficulty here, however, is that the change of peak position might reflect something about cation distribution and hydration since it is at such low resolution. I personally believe Michael's explanation, and we are thinking of including it in some discussion about the linkage problem in the paper, and make Michael a co-author. It seems to me that the paper is a good opportunity to publish very briefly some of the consequences of the model which would take a long time to write up and explain fully. The full papers by Len and Michael will appear in due course, but I think we ought to get the ideas out now.

The last paragraph answers your letter of 29 April.

Now to reply to your letters of 27 April.

Letter 1

Much of this doesn't need a reply in view of the Fourier syntheses later sent you. We believe that the clipped core particles are not very

different from the intact because of:-

1. The sedimentation values
2. The DNase I patterns
3. Close correspondence in crystal structure.

I think you tend to make too much of the Stokes' diameter. A value of 105 Å would fix the third dimension of 110 x 100 Å at about 80, but we know the answer is 57 Å. I therefore discount it for the additional very good reason that if one follows through the same hydrodynamic data one arrives at an axial ratio of 12:1 (Olins et al., Nucleic Acids Res. Dec. 1976 Table 2). So it is the old story of not being able to distinguish between hydration and frictional effects.

We don't despair of solving the structure by isomorphous replacement, but it is absolutely necessary when dealing with these large asymmetric units to solve the packing first so that the difference Pattersons of the heavy atom derivatives can be more easily interpreted. (This is what we did for the TMV protein disk.) However, I feel that until the crystals get larger and/or one produces a smaller unit cell, it might be better to proceed to exploit the present crystals, e.g. by staining the DNA with uranyl or trying to match out the protein with sucrose, etc. None of this will tell us about kinking versus bending: for that we must get higher resolution. I am hopeful that we will.

About the Zachau reconstituted material. I did write to you earlier about Jean Thomas' experiments in this direction, and mentioned that John Finch's e.m. pictures showed the same curly-coily appearance as the ordinary nucleofilaments. I agree with you that this is high on the priorities when it comes to thinking about higher order structures and that is why I have been urging Jean to go on with this. I think we will try to draw fibres of the material.

Thank you also for the copies of your letters to Keller and to Worcel. They are all very much to the point. I just don't think that Keller's results compel one to one turn per nucleosome since we don't know how H1 interacts with DNA. Presumably you will have seen an earlier paper by Renz which talks about cooperative binding of H1 to DNA under appropriate salt conditions, and the earlier papers by Maxine Singer that the binding is dependent on the superhelicity. About Worcel, I have the same difficulty as you in trying to understand his language but one can't expect it to be couched in the language of coiled coils and the like. These are things I learnt at your knee, so to speak, and I don't think a biochemist would struggle with the alpha keratin papers. It seems, however, that other people do understand what Worcel is trying to say, and it would be a hopeless job to try and rewrite his manuscript. Doubtless you will express the whole thing succinctly at some point. However I must say I have doubts about its correctness. My feeling now is that the nucleosome core particle is a distinct entity and that the linker region need not at all continue with the same curvature as the core particle. As I wrote in an earlier letter, it may be that the linker, which is now possibly only 40 base pairs in rat chromatin, plus H1, might be there to provide the necessary

which behave like distinct
articulation between the nucleosome cores packets. If so, there is no reason to postulate a continuous distribution of equal curvature on the DNA.

Letter 2

Thanks for all your points about histone packing. My diagrams were intended only to be very schematic, and indeed I have considered histone packing models in 3-D. Michael Levitt has done quite a number of more precise calculations using volumes of the histones but the trouble is there are just too many possibilities. I was mystified by your comments at the bottom of page 2 and top of page 3. I couldn't see how one could think otherwise than that the particles could get closer by adopting an approximately helical packing. I assume that you therefore mean that the "1½" turn structures pack as they would if they were complete two turn structures forming a continuous helix.

Your point about the crosslinking is well taken, and I have written something in my memo of 19 May about this.

We haven't tried using Roger's method of trimming since we have been struggling for months with the proteolysis, but you are right that such treatment might produce rather more homogeneous particles. Roger had said he was going to send us the exact details but he didn't, except for the Royal Society manuscript. I am asking John Finch to check with him.

Thank you also for the copy of your letter to Renz. Indeed he had sent us copies of both papers and I have discussed these with John and Linda. There is a distinct difference in results on the nucleofilaments here. In Cambridge, when nucleofilaments are prepared by lysing nuclei into 0.2 mM EDTA or indeed dialysing into the same solution, the filaments are curly-coily and, sometimes the coiling is sometimes severe enough to give the impression of 200 Å fibres, but we don't see distinct beads. On the other hand, in Tubingen, they see beads (cf. Figure 5b of the PNAS paper and Figure 1d of the Hozier manuscript). In 70 mM salt, Linda sees mostly beads and a small proportion of fibrous material which look somewhat like the Tubingen Figure 5a. There are often helical clumps of about 200 Å but they are never regular or definite enough to believe that they are distinct entities. In Figure 5a of Tubingen, they tend to see fibres only, but this could be because the material has come off a sucrose gradient. On the general question of knobbly fibres, the fact is that we have seen these for many years since the solenoids are often irregular enough to give this appearance, and it may amuse you to know that I coined the word "superbeads" about a year ago when we were considering whether these were regular enough. The only place where they look consistent enough is indeed in metaphase chromosomes of Aspergillus which were prepared by Ron Morris and which John looked at. There is no doubt that the fibre width is about 300 Å but the fibres are so convoluted in places that they give the appearance of producing little bubbles and, as John will tell you, we did consider a superbead model for this, but without any physico-chemical or biochemical evidence, one would not put this forward.

I am sorry I shall miss the meeting, but it turns out that I could not have come in any case as my eldest son has developed glandular fever and has been going through a bad patch. I look forward to hearing from you via John, Len and Jean, and seeing you in Aarhus.

Yours ever,

Aaron

A. Klug

Encs.